Polymerization of the Through-pores in HPLC Columns for Enhanced SEM based Assessment of Packing Order

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INTRODUCTION

Recent developments in chip-based column technology, whereby plate heights corresponding to the domain size can be obtained,[1] suggest that it might also be possible to improve the lower minimal plate height limit in packed column HPLC ($h \approx 2d_p$), down to lower values if feasible improved packing procedures can be developed.

In order to allow enhanced understanding of the order in packed HPLC columns, in this work a methodology for immobilizing silica particles is developed based on the polymerization of a monomer and a cross-linker in the interstitial pores of HPLC columns. Subsequent mechanical cutting allows scanning electron microscopy (SEM) based imaging of cross sections of the packed bed over the entire length of the column.

In this way the packing efficiency of in-house packed and commercial HPLC columns comprising the same packing material can be compared. The methodology is developed for native silica used in e.g. hydrophilic interaction liquid chromatography and the information obtained is cross-referenced with external porosity measurements obtained via inversed size exclusion approach.[2]

EXPERIMENTAL

Columns:

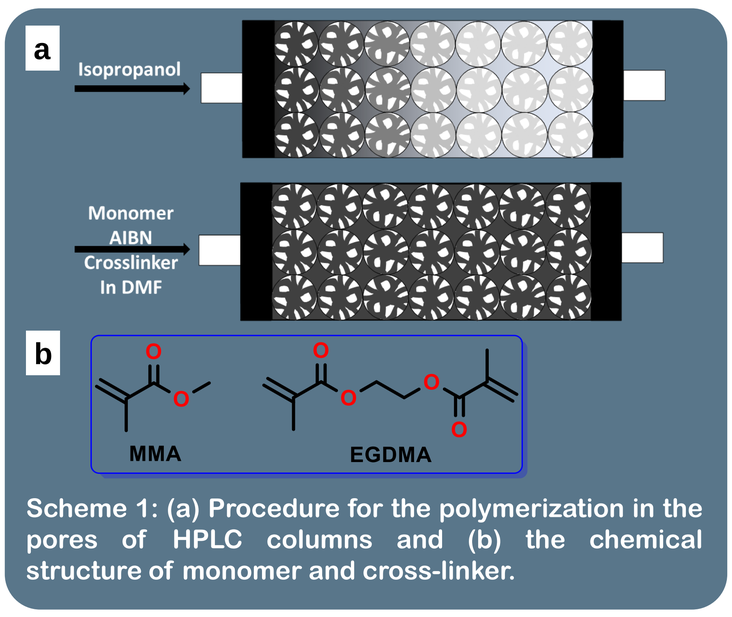
- Home-made column (4.6 x 150 mm, d_p = 5.0 µm) was packed by slurry packing process. Silica gel was obtained from an unpacked commercial column (Zorbax RX-SIL). Both the slurry and packing solvent was methanol
- Commercial column (4.6 x 150 mm, d_p = 5.0 µm, Zorbax RX-SIL).

Methodology:

- Total porosity (ε_t) values were assessed from the elution time of an unretained marker (acenaphthene) and through pycnometry using tetrahydrofuran (THF) and dichloromethane (DCM) as pure liquids.
- External porosity (ε_e) were measured experimentally by inversed size exclusion chromatography (ISEC) using a set of 12 polystyrene standards with different molecular weight.
- Van Deemter curves were constructed for evaluating column efficiency, .
- The silica gel was immobilized by the cross-linking polymerization in pores of columns (scheme 1).

Apparatus:

- All HPLC experiments were performed on Agilent 1100 HPLC system with VWD detector.
- LKB Bromma 2248 HPLC pump was used for filling polymeric solution into chromatography columns.
- The morphology of polymeric monolith was analyzed by a SEM (FEI Quanta 200F)



Scheme 1: (a) Procedure for the polymerization in the pores of HPLC columns and (b) the chemical structure of monomer and cross-linker.

RESULTS & DISCUSSION

Column efficiency

Figure 1 represents the plate height curves obtained on the two column types. Van Deemter plots for the last eluting compound in the chromatogram were constructed (guanine (k"=10.62 for the home-made column and k"=13.11 for the commercial column)). The minimum plate heights (H_{min}) obtained for home-made column was higher than that on commercial column.

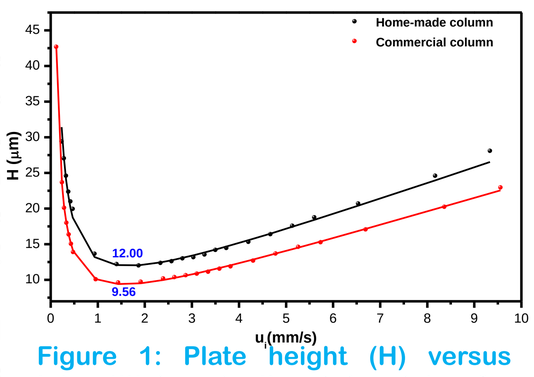


Figure 1: Plate height (H) versus interstitial velocity (u_i) plots for guanine on home-made column (black) and commercial column (red).

Column Porosity

Total porosity values (ε_t) were calculated for two columns from the elution time of their respective t_0-makers (acenapthene) using a mobile phase composition of ACN/H_2O=92/8 vol%/vol%.

$$\varepsilon_T = \frac{V_0}{V_G} = \frac{t_0 \cdot F}{V_G}$$

As the ε_t values obtained from elution volume depend to some extent on the composition of mobile phase,[3] additionally, ε_t values were also determined via pycnometry.

The external porosity ε_e were measured by ISEC experiments (Figure 2). All obtained values are displayed in table 1, suggesting the "true" or "maximum" column dead volume and external porosity. This reveals the different packing quality.

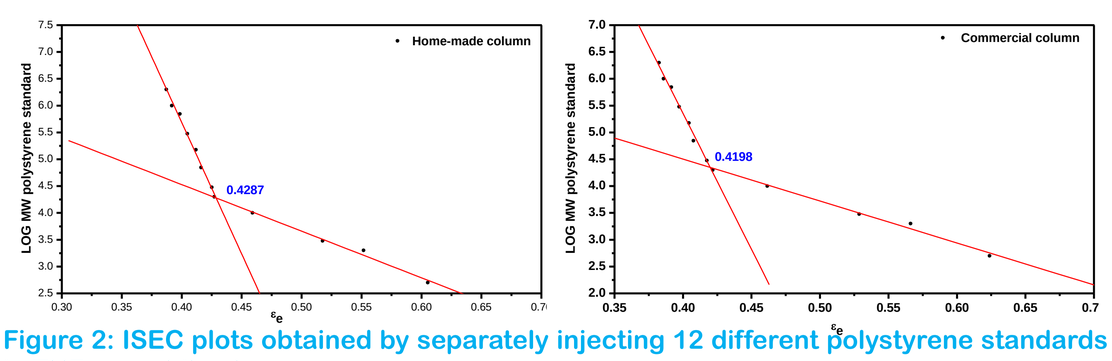


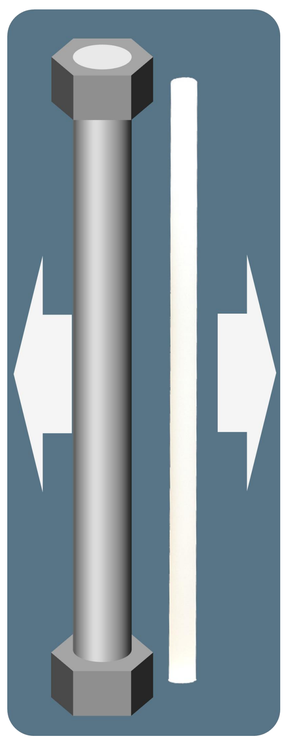
Figure 2: ISEC plots obtained by separately injecting 12 different polystyrene standards in THF as mobile phase

Table 1: Porosity of the evaluated columns.

Columns	Dimensions (mm)	d_p (µm)	ε_T (VD)	ε_T (Pycno)	ε_e (ISEC)
Home-made column	4.6 x150	5	56.98%	65.70%	42.87%
Commercial column	4.6 x150	5	57.31%	67.20%	41.98%

Morphology of polymeric monolith

The immobilization of silica particles was achieved by crosslinked polymer. The cross-linking polymerization of methyl methacrylate (MMA) and ethylene glycol dimethacrylate(EGDMA) was performed in the pores of chromatography columns by radical polymerization. Subsequently, the polymeric monolith was extruded from stainless steel columns and pending for SEM measurements. The morphology of wall and cross-section of polymer monolith shown in Figure 3.



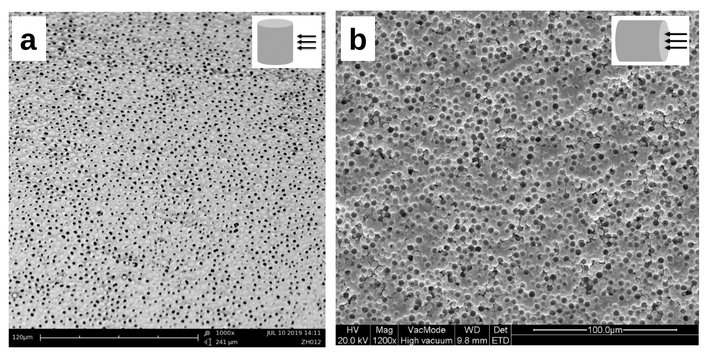


Figure 3: SEM images of (a) wall, and (b) cross-section with silica gel, of polymeric monolith.

The area percentages of silica gel in cross-section of the monolith were processed by Imagj software (Figure 4). To minimum the errors, four SEM micrographs for each monolith were processed and the results are displayed in table 2.

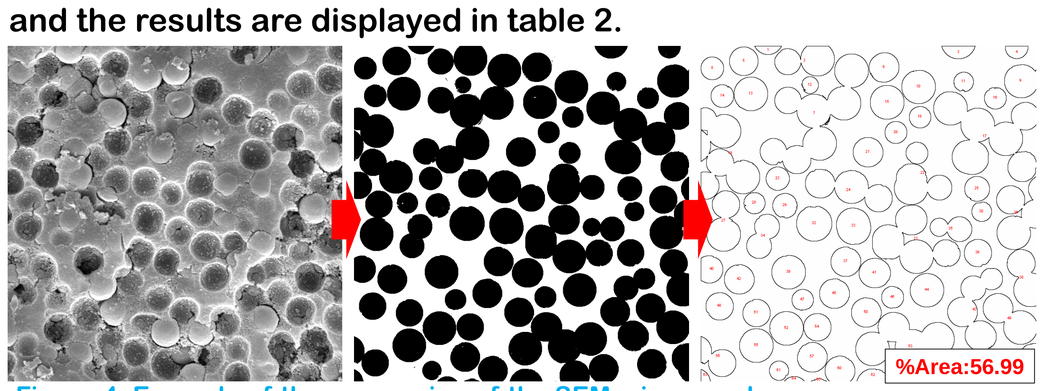


Figure 4: Example of the processing of the SEM micrographs.

Table 2: Area percentages of silica gel in cross-section of evaluated columns.

Columns	Slice 1	Slice 2	Slice 3	Slice 4	Average
Home-made column	59.41%	52.86%	61.25%	56.99	57.63%
Commercial column	61.25%	65.59%	63.85%	62.77	63.37%

CONCLUSION

- **Van Deemter measurements revealed a worse efficiency for the home-made column, as well as a larger external porosity, suggesting a lower packing quality.**
- **The lower area percentage of the measured silica on the cross section of the home-made column shows the lower packing density of silica gel**
- **To the best of our knowledge, this is the first study allowing imaging of the quality of a packed bed via a particle immobilization approach.**

REFERENCES

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Z. Hou acknowledges the Research Foundation-Flanders (FWO), BELGIUM